Selective deficiency in protein kinase C isoenzyme expression and inadequacy in mitogen-activated protein kinase activation in cord blood T cells

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The biochemical basis for the reduced lymphokine production by neonatal T cells compared with adult T cells remains poorly defined. Previous studies have raised the possibility that neonatal T cells could be deficient in their ability to transmit signals via protein kinase (PK) C. We now report that while PKC-dependent activation of the mitogen-activated protein (MAP) kinases, c-Jun N-terminal protein kinase and the extracellular signal-regulated protein kinase (ERK)1/ERK2, was deficient in cord blood T cells compared with adult blood T cells, marked activation of the MAP kinases in cord blood T cells was achieved via PKC-independent means. Consistent with a deficiency in the signalling capability of PKC, cord blood T cells were selectively deficient in the expression of PKCβI, ϵ, θ and ζ. Stimulation of cord blood T cells resulted in a time-dependent increase in PKC expression, with increases detectable by 4 h. This was accompanied by an enhancement in MAP kinase activation via PKC-dependent means. These novel data suggest that an inadequacy in PKC-MAP kinase signalling may be responsible, at least in part, for the phenotype of cord blood T cells.

Key words: cord blood T cells, mitogen-activated protein kinase, protein kinase C.

INTRODUCTION

Neonatal T cells are functionally deficient in their ability to produce a number of lymphokines [1–7] and the biochemical basis for this is not understood. While at least 40% of adult blood T cells express the CD45RO isoform of the common leukocyte antigen, studies in cord blood T cells, a surrogate for neonatal T cells, have shown that greater than 97% of these cells express CD45RA [8]. Although differences in surface phenotype can contribute to the hyporesponsiveness of cord blood T cells, it is unlikely that these differences are the only causes for the hyporesponsiveness of cord blood T cells. Thus adult T cells of all subsets consistently produce higher levels of interferon (IFN) γ and tumour necrosis factor α than the corresponding cord blood T cell subsets [3,4,6]. The difference in the level of lymphokine production between cord and adult T cells was still evident when the cells were stimulated with PMA and A23187 [6], which directly activate protein kinase (PK) C and the Ca^{2+} signalling pathway respectively. This implies that the reduced ability of cord blood T cells to produce lymphokines is due, in part, to deficiencies in intracellular signalling.

PKC is composed of a family of at least 11 members which are divided into classical (α, βI, βII and γ), novel (δ, ϵ and θ) and atypical (ζ, ι/λ, μ or PKD) isoenzymes [9]. Each isoenzyme can have distinct as well as overlapping roles [9]. PKC has been reported to regulate the activities of the mitogen-activated protein (MAP) kinases. For example, depletion of PKC inhibits the activation of the extracellular signal-regulated protein kinase (ERK)1 and ERK2 [10], and in T cells, PKCα and PKCϵ have been reported to be required for the activation of the ERK1/ERK2 modules, whereas PKCθ is required for the activation of c-jun N-terminal protein kinase (JNK) [11,12]. The PKC-MAP kinase signalling pathways play crucial roles in regulating T cell lymphokine production. Thus antisense-induced repression of PKCα expression [13] and inhibition of MAP kinase activation block T cell lymphokine production [14–19]. T cells from PKCθ-deficient mice produce less interleukin 2 than cells from wild-type animals [20], and splenic T cells from JNK-deficient mice are also compromised in interleukin 2 production [21]. Similarly, murine T cells, which express a dominant negative mutant form of the p38 MAP kinase, produce less IFNγ than wild-type T cells [22].

The failure of PMA and A23187 to overcome the deficiency in lymphokine production in cord blood T cells [6] prompted us to investigate whether cord blood T cells were deficient in their ability to signal via PKC. We now report that stimulation of cord blood T cells with PMA evoked a lower level of ERK1/ERK2 activation compared with adult T cells. PKC-dependent activation of JNK was also deficient. However, strong activation of the MAP kinases was observed if the cord cells were stimulated via PKC-independent means. Our data show for the first time that cord blood T cells express lower amounts of a number of PKC isoenzymes than adult blood T cells. These data strongly suggest that reduced expression of a number of PKC isoenzymes in cord blood T cells was responsible for the deficient coupling of

Abbreviations used: ERK, extracellular signal-regulated protein kinase; GST-jun (1–79), a recombinant protein fragment of c-jun coupled to glutathione S-transferase; IFN, interferon; JNK, c-jun N-terminal kinase; MAP kinase, mitogen-activated protein kinase; PHA, phytohaemagglutinin; PK, protein kinase.

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the MAP kinases to PKC, resulting in the reduced capacity of these cells to produce lymphokines.

EXPERIMENTAL PROCEDURES

Materials

RPMI 1640 and Hanks' Balanced Salts Solution were obtained from Cell Image, The Women's and Children's Hospital (Adelaide, South Australia). Foetal calf serum was purchased from MultiSer, Biosciences Pty Ltd, Australia. Lymphoprep was obtained from Nycomed (Oslo, Norway). Anti-PKC isoenzyme antibodies (sc-8393, 212, 209, 937, 210, 214), anti-ERK2 (sc-154) and anti-JNK1 (sc-474) antibodies were purchased from Santa Cruz Biotech. (Santa Cruz, CA, U.S.A.). The anti-ACTIVE ERK antibody was obtained from Promega (Madison, WI, U.S.A.). Reagent Plus enhanced chemiluminescence reagent was purchased from NEN Life Science Products (Boston, MA, U.S.A.). [γ-^{32}P]ATP (specific radioactivity 4000 Ci/mmol) was obtained from Geneworks, Adelaide, South Australia. Reagents for flow cytometry were purchased from BD-Pharmingen (San Diego, CA, U.S.A.).

Preparation of T cells

Umbilical cord and adult blood were obtained from mothers with normal deliveries and from healthy volunteers respectively, according to the institution's guidelines on human ethics. To obtain mononuclear cells, the blood was layered on Ficoll-Hypaque (density of 1.114 g/ml). T cells from this leukocyte fraction were prepared using Lymphoprep essentially as described previously [23]. Briefly, the mononuclear leukocyte fraction was incubated in plastic tissue culture dishes and the non-adherent lymphocyte fraction was collected and passed through two cycles of nylon wool columns [23]. The T cells that were eluted were at least 95% pure and 99% viable.

Preparation of cell lysates

Cells were lysed by incubation in 300 μl of buffer A [20 mM Hepes, pH 7.4, 0.5% (v/v) Nonidet P40, 100 mM NaCl, 1 mM EDTA, 2 mM Na_3VO_4, 2 mM dithiothreitol, 1 mM PMSF, and 10 μg/ml each of leupeptin, aprotinin, pepstatin A and benzamidine] for 2 h (4 °C) with constant mixing [38]. After lysis, cell debris was sedimented (12000 ***g*** for 30 s) and the protein content of the soluble fractions was determined by the Lowry's protein estimation method. Samples were stored at −20 °C until assayed.

ERK1/ERK2 activation

To assay for ERK activity, equal amounts of lysate protein were precleared with Protein A–Sepharose and ERK2 was immunoprecipitated using an anti-ERK2 antibody, followed by the addition of Protein A–Sepharose. Kinase activity was assayed using myelin basic protein as a substrate, as described previously [24]. Phosphorylated substrate was resolved by SDS/16%-PAGE and the radioactivity associated with the substrate was quantified using an Instant Imager (Packard Instruments, Canberra, Australia).

JNK assay

A solid phase assay was employed for the determination of JNK activity [24]. In preparations where an insufficient number of cells was isolated, lysates from 2–3 experiments were pooled as appropriate, the protein content of the resultant mixtures was obtained and the appropriate amount of protein was then loaded on to GST-jun (1–79)–glutathione Sepharose, where GST-jun (1–79) is a recombinant protein fragment of c-jun coupled to glutathione S-transferase. The data obtained from these mixtures were treated as though they were from a single experiment. Phosphorylated GST-jun (1–79) was resolved by SDS/12%-PAGE and the radioactivity associated with GST-jun (1–79) was quantified as described above.

Western blot

To determine PKC expression, cells were sonicated in buffer containing 2% Triton X-100 [10]. Equal amounts of soluble proteins (usually 50 μg) from each pair of adult/cord blood T cell sample were subjected to Western blot analysis. Immediately after transfer, the proteins were stained with Ponceau S [0.1% in 5% (v/v) acetic acid], lightly destained with H_2O, and visual checks were conducted to confirm the even loading and transfer of proteins in all the lanes of the gels. The destaining step greatly enhanced the signal-to-noise ratio of the stained protein bands. Blots that showed dissimilar protein levels between the lanes were discarded. Each blot was probed with an isoenzyme-specific anti-PKC antibody and detection was achieved by enhanced chemiluminescence [10]. The intensity of the bands from each pair of adult/cord samples were determined by densitometric scanning (Image Quant scanner, Molecular Dynamics, Sunnyvale, CA, U.S.A.) and the results were expressed as a percentage of adult band intensity. To determine the levels of ERK1/ERK2, JNK1 and dual-phosphorylated ERK1/ERK2 in the lysates, equal amounts of proteins (50 μg) were subjected to Western blot analysis using an anti-ERK2, anti-JNK1 and anti-ACTIVE ERK antibody respectively. Again, only blots which showed similar amounts of proteins between the different lanes were used. Where appropriate, the intensity of the bands was determined by densitometric scanning.

FACS analysis

T cells (1×10^6) were centrifuged at 600 ***g*** for 5 min at 4 °C, washed once with isoton, and resuspended in 50 μl of either antibody mix (20 μl of cold isoton, 10 μl of anti-CD3-PC5, 10 μl of anti-CD45RA-FITC, 10 μl of anti-CD45RO-phycoerythrin) or control antibody mix (10 μl of cold isoton, 40 μl of γ_1/γ_{2a} Simultest Control) for 20 min at 4 °C. The cells were then washed twice with 2 ml of cold isoton, fixed with 1% formaldehyde in isoton, and analysed on a Becton Dickinson FACScan using Cell Quest software.

Statistical analysis

Where indicated, statistical analysis was performed using analysis of variance (ANOVA), Student's unpaired *t*-test or Bonferroni's multiple comparison test.

RESULTS AND DISCUSSION

As reported previously [1,6], cord blood T cells were found to produce less IFNγ and lymphotoxin than adult blood T cells (results not shown). Compared with adult T cells, stimulation of cord blood T cells with anti-CD3 and anti-CD28 antibodies to mimic T cell receptor and CD28 stimulation resulted in minimal JNK activation (Figure 1a). Since cord blood and adult blood T cells express similar levels of both CD3 and CD28 [3], the data in Figure 1(a) suggest that cord blood T cells were unable to transmit signals downstream of the T cell receptor–CD3 complex and CD28 to the JNK module. To investigate this further, T cells

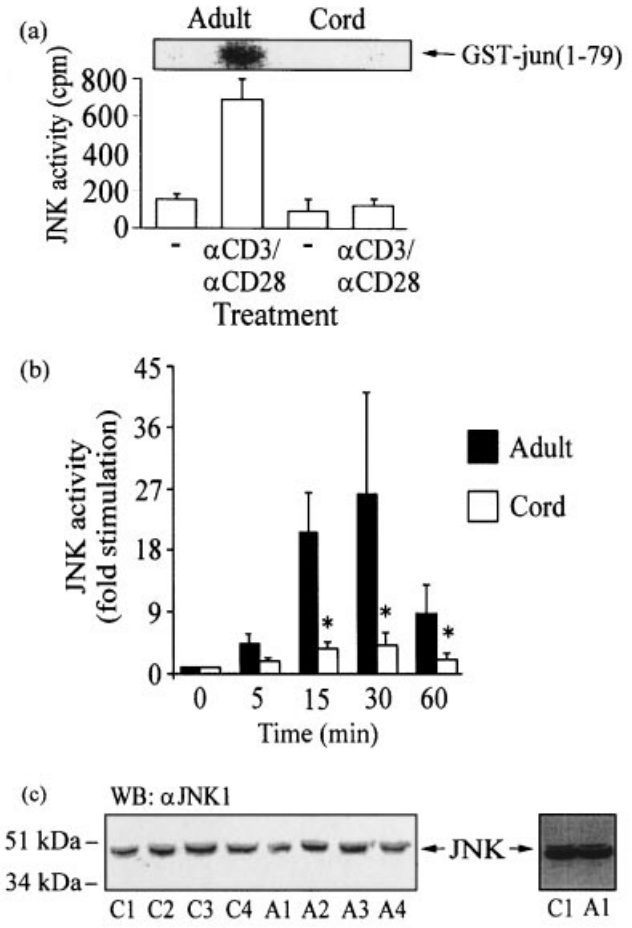

Figure 1 Reduced activation of JNK in cord blood T cells

Pairs of cord and adult blood T cells (2×10^7) were incubated in the absence (—) or presence of anti-CD3 and anti-CD28 antibodies for 30 min (**a**) or with PMA (50 nM) and A23187 (0.1 μM) for up to 60 min (**b**). After lysis, equal amounts of lysate protein from each sample were applied to GST-jun (1–79)–glutathione Sepharose and the activity of JNK was determined. Results are depicted as (**a**) a representative autoradiogram (upper panel) and pooled data (lower panel) from two experiments (mean c.p.m. ± range), or (**b**) fold stimulation over kinase activity in non-stimulated cells (means ± S.E.M. of 6 experiments). (**c**) Equal amounts of lysate protein from four batches of unstimulated cord (C1–C4) and adult (A1–A4) T cells were also subjected to Western blot analysis to show that cord and adult T cells express similar amounts of the type 1 JNK (46 kDa). The right-hand panel shows a Western blot of C1 and A1 but with the film being exposed for a longer period. *Significance of difference in kinase activity between cord and adult cells, $P < 0.05$; WB, Western blot.

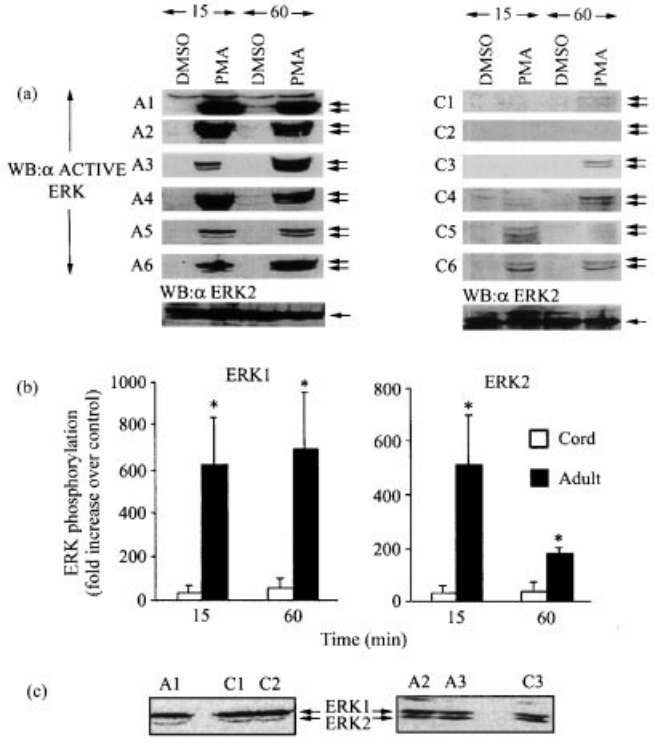

Figure 2 Reduced activation of ERK1/ERK2 in cord blood T cells

Pairs of cord and adult T cells were incubated in the presence of PMA (50 nM) or an equivalent amount of DMSO (0.02% by vol.) for 15 or 60 min. Equal amounts of lysate protein were Western-blotted using an anti-ACTIVE ERK antibody. (**a**) Results for six pairs of adult (A1–A6) and cord (C1–C6) samples are shown. Each pair of adult and cord samples was electrophoresed on the same gel. Double arrows indicate phosphoERK1 and phosphoERK2. A1 and C1 were stripped and reprobed with anti-ERK2 antibody (bottom panel). Single arrow indicates ERK2. (**b**) PhosphoERK1 and phosphoERK2 bands for all the experiments were scanned and the results (means ± S.E.M.) of nine adult and 11 cord samples are expressed as fold stimulation over control (DMSO) at 15 or 60 min. (**c**) Unstimulated cord (C1–C3) and adult (A1–A3) T cells express similar amounts of ERKs. A1, C1 and C2 were electrophoresed on one gel and A2, A3 and C3 on another. *Significance of difference in ERK phosphorylation between cord and adult cells in (**b**), $P < 0.005$; WB, Western blot.

were incubated with PMA and A23187 to stimulate the activity of JNK. Previous studies have demonstrated that receptor-independent activation of JNK requires a combination of PMA and A23187 [11]. Although JNK activation was detectable in PMA- and A23187-stimulated cord blood T cells, this was less than that observed in adult T cells (Figure 1b). The possibility that the above results were due to a reduced amount of JNK in cord cells was considered unlikely since similar amounts of the 46 kDa (type 1) form of JNK were found in unstimulated cord and adult T cells (Figure 1c, left panel). A minor immunoreactive band migrating with a molecular mass of approx. 48 kDa was also apparent in the T cells, but this required a longer exposure of the film (Figure 1c, right panel). The reduced level of MAP kinase activation was not restricted to JNK, as the data in Figures 2(a) and 2(b) show that PMA also caused a reduced level of ERK1 and ERK2 dual phosphorylation in cord blood T cells than in adult blood T cells. No differences in the content of the ERKs were detected between unstimulated adult and cord T cells (Figure 2c).

A plausible explanation for the above results is an inadequate flow of signal from an upstream regulator, such as PKC and/or Ca^{2+}, to the MAP kinases. However, a deficiency in Ca^{2+} signalling was excluded because activation of ERK1 and ERK2 by PMA alone was reduced compared with adult T cells (Figure 2). We reasoned therefore that the activities of the MAP kinases in cord cells could be increased to levels attainable in adult cells by agents which act independently of PKC. Studies in Jurkat and other cell-types have shown that anisomycin up-regulates the activity of JNK in a PKC-independent manner [11] and pervanadate causes the activation of ERK1/ERK2 [25]. Although pervanadate can stimulate the ERK1/ERK2 pathway through activation of phospholipase C γ [26], such an effect in cord blood T cells is unlikely because pervanadate caused minimal production of inositol trisphosphate in these cells [27]. Current evidence suggests that pervanadate acts predominantly via a compound inhibitory effect on phosphatase 2a [28] and on an uncharacterized tyrosine-specific phosphatase [28], which could

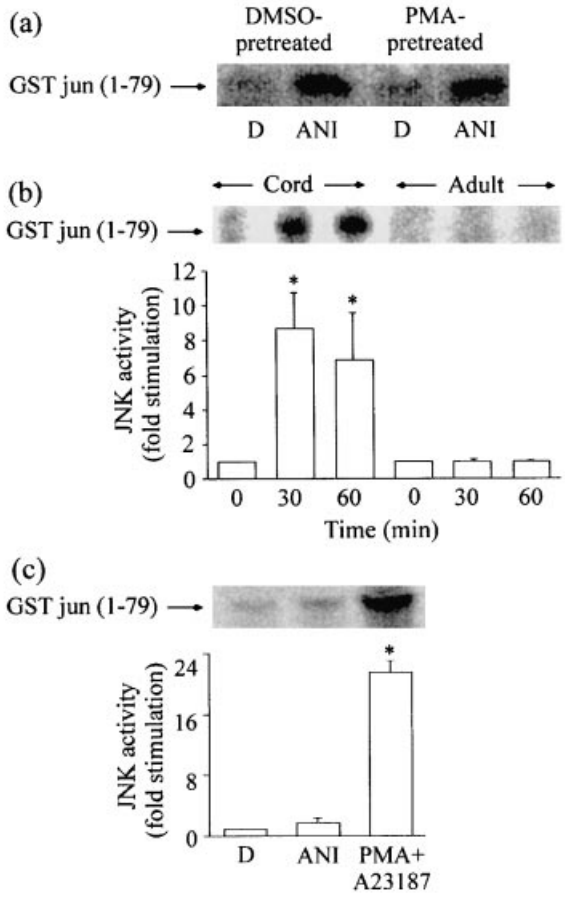

Figure 3 Anisomycin stimulated the activity of JNK in cord blood T cells in a PKC-independent manner

(**a**) Cord blood T cells were preincubated with PMA (1 μM) or an equivalent amount of DMSO (0.1% by vol.) for 24 h before being stimulated with anisomycin (ANI) (2 μg/ml in DMSO) or DMSO (D) for 30 min. The cells were lysed and the activity of JNK was determined. Results (autoradiogram) are representative of three separate experiments. (**b**) Pairs of cord and adult T cells were incubated with anisomycin for 30 or 60 min, lysed and the activity of JNK was determined. A representative autoradiogram and pooled data (means $\pm$ S.E.M.) from three experiments are shown. (**c**) In some experiments, adult blood T cells were incubated with anisomycin or PMA and A23187 for 30 min and the activity of JNK was determined. *Significance of difference in JNK activity between the presence and absence of anisomycin in cord blood T cells (**b**), and between control and PMA- and A23187-stimulated adult T cells (**c**), $P < 0.005$.

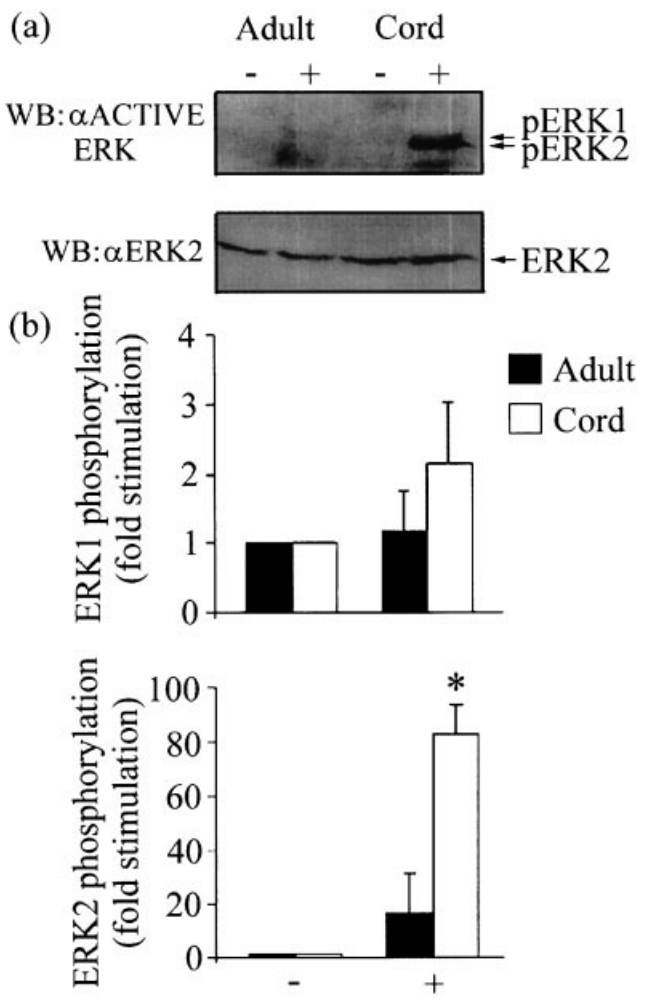

Figure 4 Pervanadate stimulated the phosphorylation of ERK2 in cord blood T cells

Pairs of cord and adult blood T cells were incubated in the presence (+) or absence (−) of pervanadate for 30 min, lysed and ERK1/ERK2 dual-phosphorylation was determined by Western blot analysis. (**a**) A representative blot is shown for phosphorylated ERK1/ERK2 (upper panel). The blot was stripped and reprobed with anti-ERK2 antibody (lower panel). (**b**) Phospho-ERK1/ERK2 bands were subjected to densitometric scanning and the results (means $\pm$ S.E.M.) of three pairs of cord and adult samples are shown. Pervanadate was prepared by mixing equal volumes of H_2O_2 (0.1 M) with Na_3VO_4 (0.1 M) and was left at room temperature for 20 min before use. *Significance of difference in ERK2 phosphorylation between control and pervanadate-stimulated cord blood T cells, and between pervanadate-stimulated adult and cord blood T cells, $P < 0.01$; WB, Western blot.

be haematopoietic protein tyrosine phosphatase [29]. We first confirmed that anisomycin acted independently of PKC in cord blood T cells. Preliminary results showed that PMA-pretreatment (0.1–1 μM) for 24 h dose-dependently reduced PKC expression, with 1 μM reducing PKCα expression by approx. 70%. The ability of anisomycin to stimulate JNK activity in PMA-pretreated cells was not reduced (Figure 3a) despite the loss of immunoreactive PKC in these cells (results not shown). This observation is consistent with data from previous studies that showed that PKC depletion did not affect the ability of anisomycin to stimulate the activity of JNK in Jurkat cells [11]. While anisomycin stimulated the activity of JNK in cord blood T cells, adult blood T cells unexpectedly showed little or no response to anisomycin (Figure 3b). Subsequent experiments show that JNK activity in the adult T cells could be stimulated by PMA and A23187, despite the deficient response to anisomycin (Figure 3c).

The data in Figure 4 demonstrate that pervanadate predominantly increased ERK2 phosphorylation in cord blood T cells. While pervanadate did not increase ERK1 phosphorylation in adult T cells, ERK2 phosphorylation was detectable, but this was substantially less than that seen in cord cells ($p < 0.01$). These data not only demonstrate that agents which acted independently of PKC were able to elicit strong activation of the MAP kinases in cord blood T cells, but also suggest a deficiency in the coupling of PKC to the MAP kinases in cord blood T cells.

PKC regulates the MAP kinase modules in an isoenzyme-specific manner in T cells. For example, PKCα and ϵ have been reported to regulate the ERK1/ERK2 modules, wheras PKCθ regulates the JNK module [11,12]. To determine whether the above results

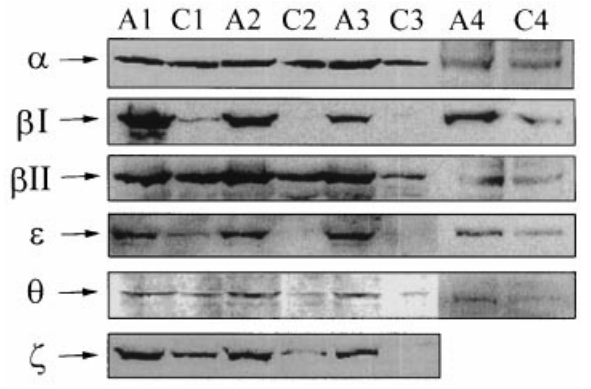

Figure 5 Reduced expression of PKC isozymes in cord blood T cells

Unstimulated T cells were sonicated in the presence of 2% (v/v) Triton X-100, centrifuged, and soluble fractions containing equal amounts of protein were subjected to Western blot analysis using isoenzyme-specific anti-PKC antibodies. Results shown are from 3–4 pairs of adult (A1–A4) and cord (C1–C4) blood T cell samples. Ponceau Red staining of proteins on the nitrocellulose immediately after electrophoretic transfer confirmed the presence of equal amounts of proteins between adult and cord samples within each pair of adult/cord samples.

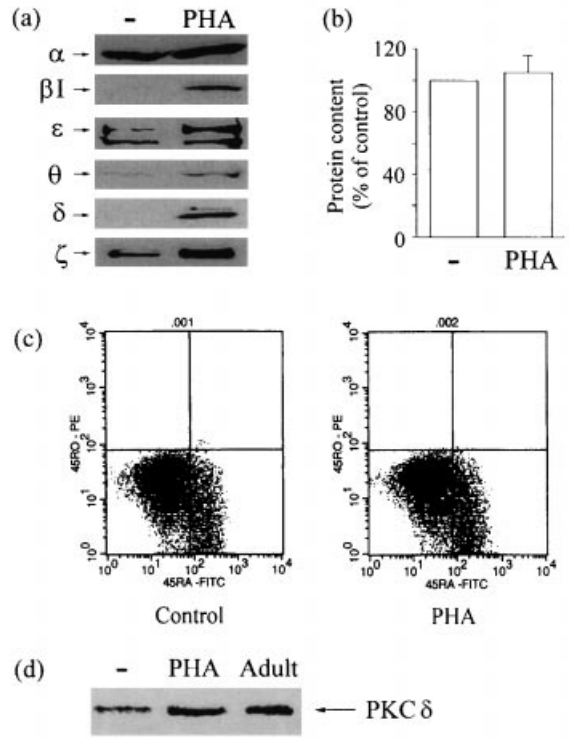

Figure 6 PHA-pretreatment increased PKC expression without affecting total cellular protein content and CD45 isoform expression

Cord blood T cells were incubated in the absence (—) or presence of PHA (1 μg/ml) for 14–16 h and the cells were sonicated and lysed or fixed. The level of PKC isoenzyme expression (**a** and **d**) was determined by Western blot analysis and protein content (**b**) was determined by Lowry's method of protein determination. Expression of CD45 isoform (**c**) was determined by FACS analysis after staining the cells with anti-CD3-PC5 and CD45RA-FITC or CD45RO-phycoerythrin antibodies. In some experiments, the level of PKCδ in PHA-stimulated cord T cells was compared with that found in unstimulated adult T cells (**d**). Results shown are representative of at least three experiments (**a**, **c** and **d**) or are the means $\pm$ S.E.M. of three experiments (**b**).

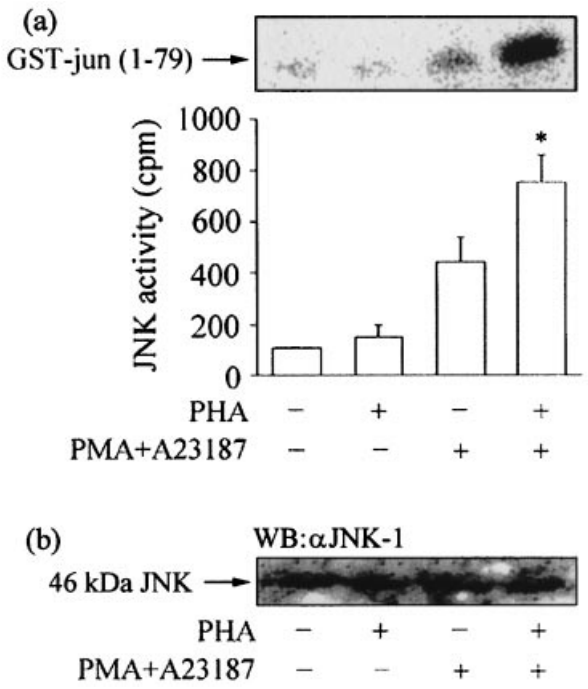

Figure 7 Enhancement of JNK activation in PHA-treated cord blood T cells

Cord blood T cells were preincubated in the absence or presence of PHA. (**a**) To investigate the effect of PHA-pretreatment on JNK activation, PHA-preincubated cells were stimulated with PMA and A23187 for 30 min, lysed and the activity of JNK was determined using GST jun (1–79) as a substrate. JNK activity is depicted as a representative autoradiogram and pooled data (means $\pm$ S.E.M.) from four experiments. The data presented in (**b**) show that the above treatments did not affect the level of the 46 kDa form of JNK in the cells. *Significance of difference in JNK activity between cells exposed to PMA and A23187, and those which had been pretreated with PHA and challenged with PMA and A23187 (**a**), $P < 0.05$. WB, Western blot.

could be due to a deficiency in the ability of PKC to transmit signals downstream to the MAP kinases, Triton X-100 soluble fractions were prepared from pairs of cord blood and adult blood T cells and each sample was subjected to Western blot analysis to assess the content of PKCα, βI, βII, ϵ, θ and ζ. Each cord blood sample was found to contain less PKCβI, βII, ϵ, θ and ζ than adult samples (Figure 5). Of these, the level of βI was markedly reduced. This was followed by PKCϵ, θ and ζ (Figure 5). PKCα was expressed at only slightly reduced levels compared with adult T cells. Densitometric scanning of the blots revealed that the content of PKCα, βI, βII, ϵ, θ and ζ in cord blood T cells (means $\pm$ S.E.M.) was 78 ± 7, 9 ± 6, 48 ± 10, 24 ± 12, 39 ± 6 and $27 \pm 10\,\%$ respectively, of that found in adult blood T cells. The data in Figure 6(d) show that the content of PKCδ in cord blood T cells was also less than that seen in adult blood T cells. Based on the demonstrated roles that PKCϵ and PKCθ play in MAP kinase activation in T cells [11,12], our data provide an explanation for the low levels of MAP kinase activation in cord blood T cells. Since equal amounts of protein were loaded for each pair of cord/adult samples, the data reflect differences in PKC:total protein ratios between the two populations of cells.

To provide further evidence that the reduced expression of PKC isoenzymes was responsible for the deficient MAP kinase activation in cord blood T cells, we investigated whether it was possible to increase the content of PKC isoenzymes in cord blood

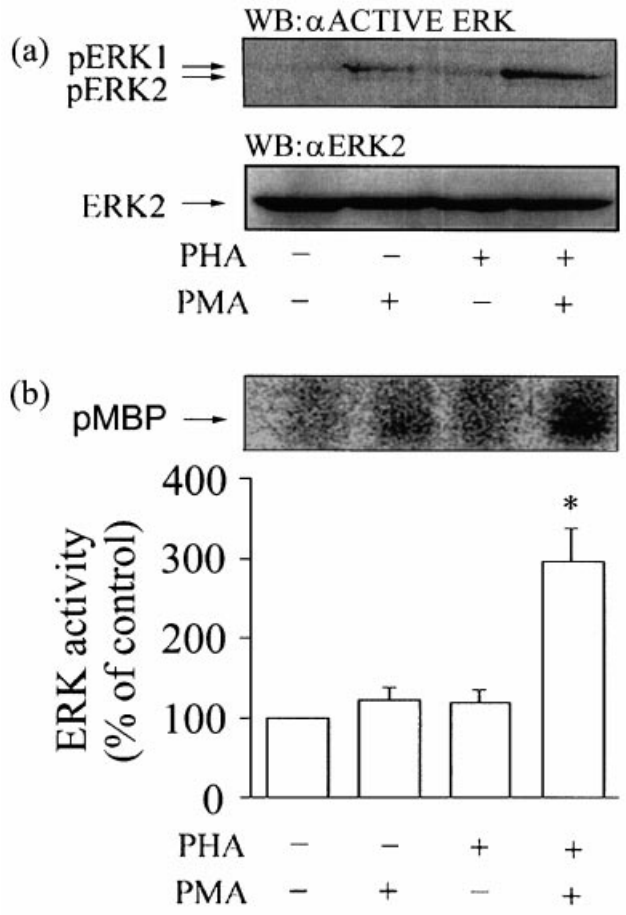

Figure 8 Enhanced activation of ERK1/ERK2 in PHA-treated cord blood T cells

Cord blood T cells were preincubated in the absence or presence of PHA before being stimulated with PMA (15 min). After lysis, dual phosphorylation (**a**, upper panel) and activity of ERK1/ERK2 (**b**) were determined by Western blot analysis and enzymic assay using myelin basic protein (MBP) as a substrate respectively. Equal loading of the lanes (**a**, lower panel) was confirmed by stripping and reprobing the blots. A representative blot of four experiments is shown for ERK1/ERK2 dual phosphorylation (**a**) and a representative autoradiogram (**b**, upper panel) and pooled data (means ± S.E.M.) of three experiments (**b**, lower panel) are shown for ERK1/ERK2 activation. *Significance of difference in kinase activity between PMA stimulation alone and pretreatment with PHA followed by PMA (**b**, lower panel), $P < 0.01$; WB, Western blot.

T cells and whether this was accompanied by enhanced MAP kinase activation. It has previously been demonstrated that incubation of cord blood T cells with phytohaemagglutinin (PHA) induced the expression of $p56^{lck}$ within a few hours of treatment [30]. We found that incubation of cord blood T cells with PHA caused a time-dependent increase in PKC isoenzyme levels within the cells. The results shown are for cells harvested at 14–16 h after the addition of PHA (Figure 6a) but increased levels of the isoenzymes were detectable at 4 h (results not shown). However, the degree of change differed between the isoenzymes (Figure 6a). All, except PKCα, exhibited at least a three-fold increase. The increase in PKCα expression was approx. 30%. PHA did not significantly affect the total cellular protein content in the cord cells (Figure 6b) or cell number (results not shown) over this time period, suggesting a selective action of PHA on PKC levels during this period. Furthermore, incubation of cord blood T cells in the presence of PHA for 14–16 h did not cause a shift in the expression of CD45RA to CD45RO (Figure 6c), implying that maturation of these cells had not occurred during this period. When the level of PKCδ in PHA-stimulated cord cells was compared with that present in adult T cells, the data show that PHA increased PKCδ in cord cells to levels found in unstimulated adult T cells (Figure 6d).

Preincubation of cord blood T cells with PHA for 14–16 h not only enhanced PMA and A23187-stimulated JNK activation (Figure 7a) but also increased PMA-stimulated dual phosphorylation of the ERKs (Figure 8a, upper panel). Enhancement of PMA-stimulated ERK1/ERK2 activity was also apparent (Figure 8b). PHA-pretreatment did not affect the expression of either the 46 kDa form of JNK (Figure 7b) or ERK2 (Figure 8a, lower panel). These results demonstrate that increased levels of PKC expression was accompanied by enhanced activation of MAP kinases and that these changes were not due to a change in CD45 isoform expression or an increase in MAP kinase expression.

A deficiency in PKC expression is likely to be a significant cause of the deficient coupling between the T cell receptor and the MAP kinases. A number of observations are consistent with this. First, PKC-dependent activation of the MAP kinases was deficient in cord blood T cells and this was not due to a difference in MAP kinase expression between cord and adult blood T cells. Secondly, when cord blood T cells were challenged with agents which acted independently of PKC, marked activation of the MAP kinases was achieved. Thirdly, cord blood T cells were deficient in a number of PKC isoenzymes, and some of these are known to regulate the activities of ERK1/ERK2 and JNK [11,12]. Finally, increased cellular content of PKC was associated with enhanced activation of the MAP kinases. Our data are thus consistent with a causal relationship between the low PKC expression and inadequacy in MAP kinase activation. The deficiency in PKC expression and MAP kinase activation also provides an explanation for the inability of PMA and A23187 to stimulate cord blood T cells to produce cytokines at levels seen in adult blood T cells [6]. This is consistent with the demonstration that PKC and the ERK1/ERK2 and JNK modules are involved in regulating T cell lymphokine production [13–21]. Thus our data provide evidence for an inherent signalling deficiency in cord blood T cells which is independent of surface receptor expression.

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